

Petrijeve mreže u biologiji

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DIVISION OF BIOLOGY

PETRI NETS IN BIOLOGY

PETRIJEVE MREŽE U BIOLOGIJI

BACHELOR THESIS

SEMINARSKI RAD

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Undergraduate Study of Molecular Biology

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1. INTRODUCTION

Living organisms are the world's most complex phenomena. Life is comprised of signaling pathways – huge signaling cascades that begin at the cell membrane with a single signal being transferred from extracellular fluid across the membrane to the cytosol, that afterwards becomes amplified and directed to the nucleus in order to change the expression of genes. The pathways lie within broad networks of proteins, which are able to influence a wide amount of various cell processes such as growth, survival, proliferation, differentiation and death. Some of these processes are essential to the cell and are therefore often changed in complex diseases such as cancer, making the research of cellular networks highly important. To enable the design of experiments used for investigating cellular networks, it is important to develop tools that can be utilized for the observation and analysis of the networks. Static regulatory networks have long been used to observe the connectivity of cellular networks and to better understand the way they operate. Nowadays, dynamic modeling of regulatory networks is used to analyze and predict the behavior of cellular signalling networks, which creates a significant emphasis on local network properties and individual biochemical reactions. The motivation for substituting static networks with dynamic modeling comes strongly from the need to illustrate network properties such as liveness, reachability and reversibility, which provides a much more detailed insight into the network.

Petri Nets are one of several methods for the modeling of dynamic networks, such as Ordinary Differential Equations, Probabilistic Boolean networks, Thomas networks, etc. They were defined by Carl Adam Petri in his dissertation thesis in 1962., with the aim to define the mathematical formalism for representing and analyzing causal systems with concurrent processes. In comparison to other methods, Petri Nets use a coarse-grained approach, somewhat relaxing the need for a very detailed amount of knowledge usually required by other methods to simulate a network. This is a strong advantage, because it results with less erroneous information in the pathway, as there is a lower possibility to choose incorrect data. Petri Nets are also more cost-efficient, because their flexibility significantly lowers the amount of network parameters that have to be obtained through wet-lab experiments prior to the simulation. In this paper I will go through basic principles of Petri Nets and the various types of their existence and also, show an example of a complex biological question regarding the thematics of PI3K/Akt pathway, that can be solved by using Petri Nets (Koch et al. 2005).

2. PETRI NET METHODOLOGY

Petri Nets can be divided into two aspects: static and dynamic. The static aspect comprises of system components (places, transitions, tokens, markings) and arcs (Fig. 1.).

Places (p) are marked by circles. They represent proteins and are given individual names during the experiment. Places are a passive system component.

Transitions (t) are shown as boxes. They enable and control interactions between proteins (places) and are used to model chemical reactions, such as synthesis, degradation and complex formation. This also means they are an active system component.

Directed arcs link places and transitions. Graphically, they are displayed as arrows. An arc always connects a place and a transition, it never connects two places or two transitions. Arcs never represent a system component, rather they are used to mark the relationship between components. In order to put emphasis on certain molecular interactions, a certain amount of weight (integer value) can be given to an arc.

Tokens represent protein concentrations. Each protein is given a certain number of tokens correlating with the concentration in which it exists in the cell. They are shown as black dots that are inscribed in places. During the simulation of the dynamic network, tokens are exchanged by places, which provides a measure of protein activity.

Dynamic properties of the system enable it to move stepwise from one state to another. Markings (m) represent the total state of the system at a particular step. They give information on the distribution of tokens in network at the current state. m_0 stands for the marking of the first step, therefore it represents the initial state of a network (Fig. 2.).

The dynamic aspect of Petri Nets comprises of two components: the transition status and the sequence of steps.

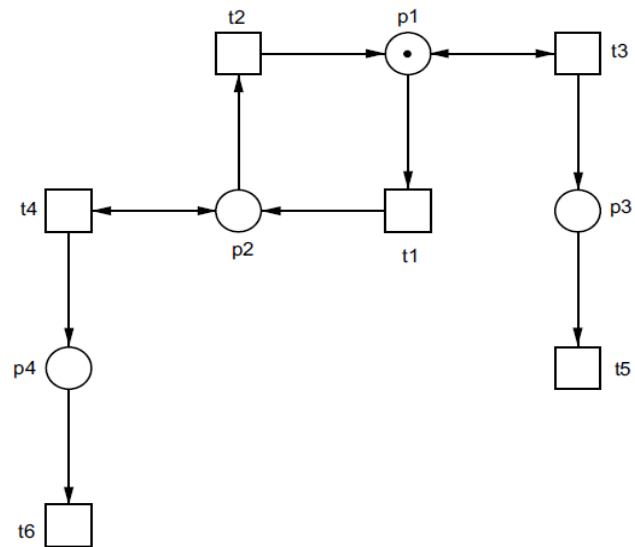


Figure 1. A Petri Net constitution.
www.en.wikipedia.org/wiki/Petri_net

Steps convert the present state of a network into a new system state upon the occurrence of a chemical reaction. After each step, tokens are exchanged between places. This is mediated by arcs, which are given a certain amount of weight, as mentioned previously. If the transition is enabled and the protein carries a large enough number of tokens that is able to exceed the weight of an arc, the tokens will be exchanged. The transition may or may not be enabled at the current marking. If it is not enabled, it will not fire and the tokens will not be exchanged.

The transition status varies from step to step. Let's assume a number of steps, i, j, z , and two transitions, m and n . In steps i and z , both types of transitions are enabled. But in step j , the transition n is never enabled. Depending on the sequence of steps, the transitions will fire in a different order, which will change the outcome of a simulation. Therefore, the dynamic properties of the network also depend on the order of steps (Koch et al. 2005).

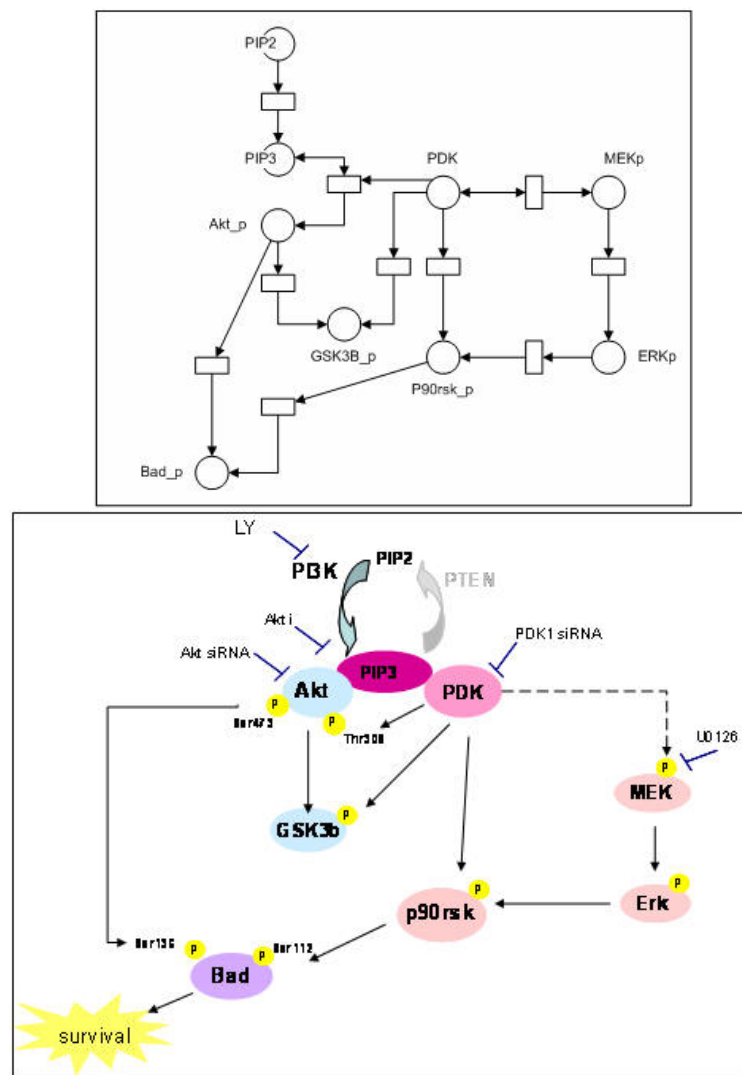


Figure 2. An example of a biological pathway and its corresponding Petri Net.

3. TYPES OF PETRI NETS

3.1. COLORED PETRI NETS

Unlike other Petri Nets that have all tokens of the same values, Colored Petri Nets obtain typed tokens that can incorporate various types of data, ranging from simple numbers to matrices and data structures. Instead of being depicted as black circles, tokens are now either shown in colors or as numbers and letters. The data value attached to a token is called a token color, and the type of value a color set. Each place has its own color set (Fig. 3.). Here, arcs become arc expressions, which upgrades them from serving as a connection between places to containing a number of operations, enabling them to evaluate and change the values of different types of passing tokens (Jensen 1987).

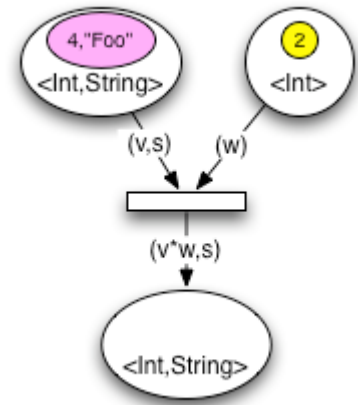


Figure 3. Colored Petri Net.
www.scienceblogs.com

3.2. HYBRID PETRI NETS

Hybrid Petri Nets are excellent for discrete behavior modeling using logical conditions (i.e. protein synthesis is either enabled or disabled). This is allowed by the introduction of a new type of transitions, which are called continuous transitions (Fig. 4.). While the firing of traditional transitions (discrete transitions) models the occurrence of an event, the firing of continuous transitions describes the flow of materials through the network. Discrete transitions are able to change the firing speed of continuous transitions, permitting a more efficient modeling of events such as molecular transport. Membrane transport is then always present as molecule diffusion through the membrane (continuous transition), but an increase in the concentrations of a transporter protein increases the total amount of transported molecule, which is made possible by a discrete transition acting upon continuous transition.

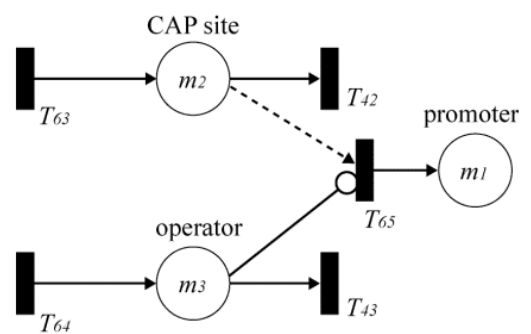


Figure 4. Hybrid Petri Net.
www.bioinfo.de

Hybrid Petri Nets also allow for inhibitory arcs, which enable transition firings only if the source place has a number of tokens less or equal to the weight of the arc, meaning that they act opposite of directed arcs. They are used to model events such as gene repression (genome.ib.sci.yamaguchi-u.ac.jp, David et al. 2001, Ghomri et al. 2007).

3.3. CONTINUOUS PETRI NETS

Continuous Petri Nets consist of two subcategories: Autonomous and Timed Continuous Petri Nets.

Autonomous Continuous Petri Nets differ from other types of Petri Nets in their approach to the evolution of time. Here, transition firing is not restricted to integer amounts. Consequently, the marking is not forced to be an integer, which means that the marking can infinitely approach to zero, but never reaches zero. This enables a better observation of network properties such as liveness, consistency and reversibility.

Timing is one of the unique qualities of Timed Continuous Petri Nets. In this type of Petri Nets, transitions are timed with the exponential probability distribution functions. Here, immediate transitions are not allowed, rather every transition has a certain delay. This concept introduces the possibility of changing the speed of transitions and delaying their offset, which makes modeling of biological reactions more precise (Recalde et al. 2007, Heiner et al. 2008).

3.4. STOCHASTIC PETRI NETS

Stochastic Petri Nets use a probability distribution to set the time of transition firing (reaction rates), which means that in each simulation reactions occur at a different rate (Fig. 5.). Theoretically, all reaction times can occur, but the likelihood depends on the probability distribution. Here, the most important task is to define the probability distribution of reaction rates. One of the ways this can be done is for the probability distribution to be influenced by the number of tokens at each place. This is used to

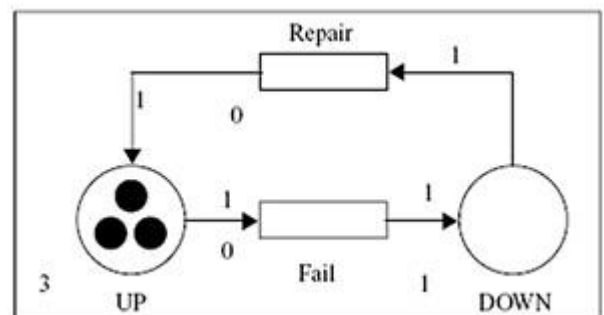


Figure 5. Stochastic Petri Net.

www.theriac.org

more closely approach the behavior of a real cellular signalling network. In the cell, an increase in the amount of protein can push the reaction balance towards products, increasing the speed of the reaction. This means that the more tokens a certain place has, the higher the probability will be for its transition to achieve a shorter waiting time (Heiner et al. 2008).

3.5. FUZZY PETRI NETS

Fuzzy Petri Nets are based on fuzzy logic systems. Fuzzy logic is used to describe the behavior of biological processes and dependencies between entities, which is usually characterised by inexact boundaries. In fuzzy logic systems, elements are not seen as being either part of a set or not but instead they are defined as being similar to elements described by a set. To make that possible, fuzzy sets are introduced to describe arbitrary entities or properties of a biological system (Fig. 6.). They are defined by the interval $[0, 1]$, where 0 stands for dissimilar, and 1 for equal, in order to allow a gradual transition from

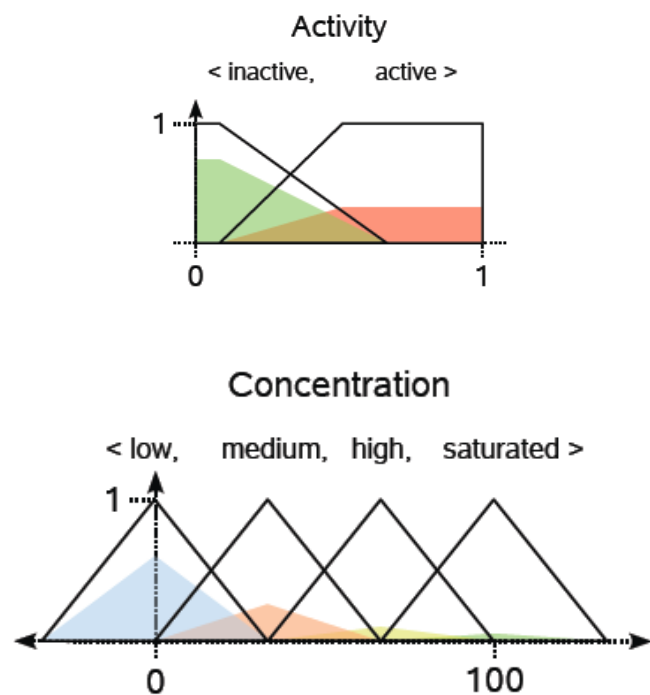


Figure 6. Fuzzy sets.
(Windhager et al. 2008)

false to true. If we used fuzzy sets to describe the concentration of a protein, there would be four fuzzy sets: low, medium, high and saturated, while if measuring protein activities, we would have two sets: active and inactive. There are never definite values – rather the protein activity or concentration changes proportionally to the amount of tokens at a place, describing the current state of the system. This is a very strong advantage when it comes to simulation of biological processes, as usually we possess only limited and inexact qualitative knowledge. Consequently, Fuzzy Petri Nets do not provide a singular answer, rather their approach is more descriptive, which again is better to describe a system not much is known about. The method is an excellent tool for building hypothetical systems, as Fuzzy Petri Nets can easily be outlined in a pen-and-paper style by creating drafts of entities and their dependencies and

describing the desired properties and effects of influences and dependencies in a natural language. This makes Fuzzy Petri Nets essential for earlier research stages in order to shed light on the underlying molecular processes of a biological system. Unfortunately, because of the aforementioned properties, Fuzzy Petri Nets are unable to return an accurate and precise result, and can be therefore used only for experiments that are more hypothetical than real (Koch et al. 2011, Windhager et al. 2008).

3.6. SIGNALING PETRI NETS

Signaling Petri Nets are the newest type of Petri Nets. Their approach is unique in a way that they do not require a large amount of data prior to the experiment. Usually, initial protein concentrations and kinetic parameters are needed to set up the dynamic network and to set the transition speed. This requires a large amount of wet-lab experimental data, which can be quite expensive. A Signaling Petri Net has two important properties to overcome this problem: the zero initial state and the network connectivity information.

We previously mentioned markings as a representation of the current state of a network. Signaling Petri Nets allow for the initial markings of a network to be zero for all places, which is called the zero initial state. As all places then have zero tokens, this does not require the knowledge of any initial protein concentrations. The systematics of a Signaling Petri Net enable the network with a zero initial state to accomplish the same accuracy as the network starting with defined initial protein concentrations. Moreover, the proteins that are known to be constitutively active can be set as 'High', enabling them to never go below a certain number of tokens, which is a weakly-defined property describing the state of a protein, but not defining it, which again does not require a knowledge of the protein concentration.

A Signaling Petri Net operates without the explicit knowledge of kinetic parameters. Here, connectivity information of the network substitutes the need for kinetic parameters. This is based on the assumption that, under the context of evolution, connectivity of biochemical networks desensitizes them to small fluctuations, which are represented by error-prone kinetic

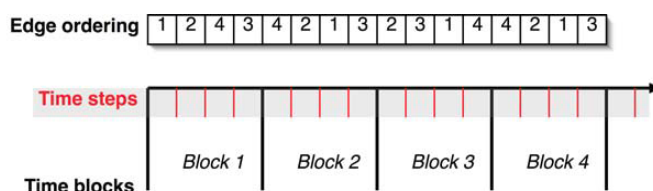


Figure 7. Connectivity information of the network.
Ruths D, et al. (2008.)

parameters. This should be a highly desired property as it ensures an offspring to remain viable despite the exact fine-tuning of individual chemical reactions. The idea is achieved by dividing the network simulation time into blocks, which are, again, split into several events (Fig. 7.). Each event is associated with one transition, and each time block has a random order of events. This makes proteins interact in a different order in each time block. In the end, when the results are averaged after a large enough number of simulations, the connectivity information of the network substitutes the need for kinetic parameters, and the speed of signal propagation throughout the network correlates the speed of signal propagation through the real signaling network (Ruths et al. 2008).

4. USING PETRI NETS IN BIOLOGY: PI3K/AKT PATHWAY

In this chapter, we will observe an example of a problem, which can be solved with the use of Petri Nets.

Protein kinase B (Akt) is one of the most prominent enzymes in the cell, because of its ability to affect a large number of substrates and efficiently mediate the signaling of cellular regulatory networks (Fig. 8.). Akt controls three most important cellular functions: growth,

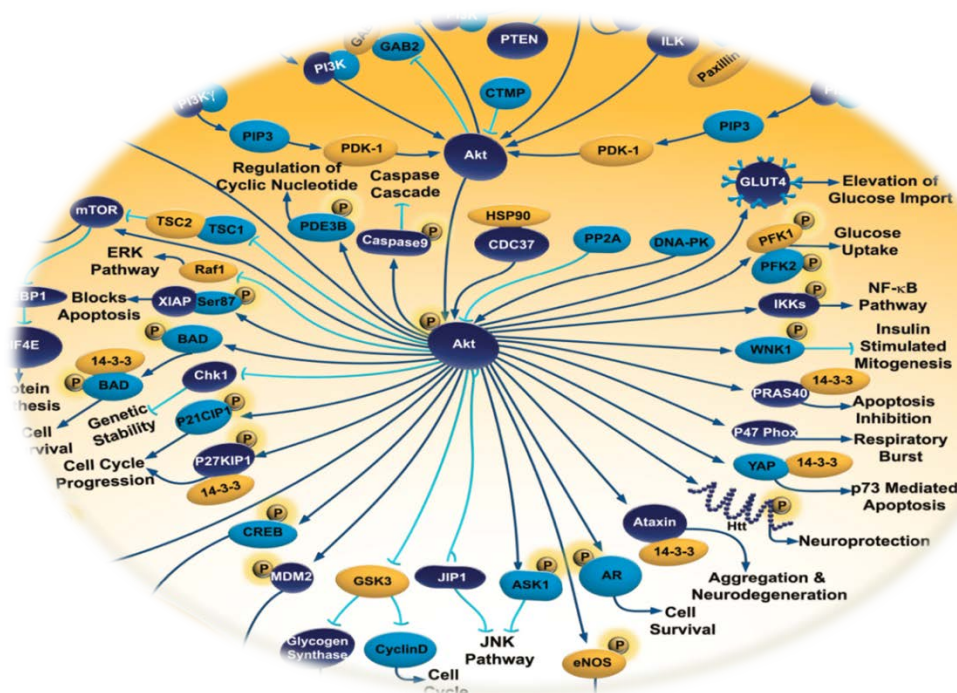


Figure 8. Akt signaling cascade.

www.docs.abcam.com

survival and proliferation. So far, the exact function of Akt has not been determined due to a conflict between two lines of research arguing over its role in the cell. The first line of research states that the activity of Akt upregulates growth, survival and proliferation, while the other line of research says that Akt downregulates these functions and rather induces senescence and apoptosis.

The activity of Akt is closely connected to a protein phosphatase PTEN (Phosphatase and tensin homolog). Moreover, Akt has shown different behavior in cells with various deletions of PTEN – there are observations that Akt induces proliferation in cells heterozygous for PTEN deletion, but induces senescence in cells homozygous for PTEN deletion (Manning BD et al. 2007). This change in the fate of the cell might be due to a difference in the activity levels of Akt, which would influence the activity of downstream targets of Akt – the Polycomb group proteins. Polycomb are transcriptional repressors of genes and are able to strongly change the gene expression profile of the cell when a shift in their activity occurs. This is a development strong enough to switch the cell from proliferation to senescence and apoptosis or vice versa (Chen Z et al. 2005.).

In order to show that the signaling of Akt completely relies on PTEN and varies under two different conditions, an experiment has to be designed that does not take into consideration only Akt, PTEN and Polycomb, but a broader network of proteins from the PI3K/Akt signaling pathway. This is where Petri Nets stand as an excellent tool for research of biological problems. Due to a large number of Petri Net types, to create a network meant to explore Akt signaling, one requires fewer or no kinetic parameters and protein concentrations. If there is no experimental data, a Fuzzy Petri Net or a Signaling Petri Net may be chosen as a model for the experiment, but if the experimental data is present, a Hybrid or a Colored Petri Net might be a better choice, so to test the hypothesis in a more detailed manner.

To build a prototype Petri Net of Akt signaling, first a number of proteins has to be chosen and set as places in the Petri Net (Fig. 9., up). In this hypothesis, there are two forms of Akt – the one that induces proliferation, and the one that induces senescence. Therefore, there will be two places representing Akt – the first describing the proliferative form of Akt, and the second describing the apoptosis-inducing Akt. Furthermore, the Petri Net has to possess two kinds of transitions in order to simulate both inhibitory and stimulatory reactions. There should be transitions which, when they fire, take a number of tokens from their final target, representing an inhibitory reaction, and also transitions which give a number of tokens to their

final target, representing a stimulatory reaction. Places should then be properly connected to the corresponding transitions with directed arcs, lastly forming a functional Petri Net that requires a number of tokens to be assigned to each place and a defined time step (Fig. 9., down).

In a nutshell, after the simulations, the results would provide a detailed pattern of protein activity, from which a conclusion could be drawn about the behavior of the PI3K/Akt signaling network. These results would then have to be confirmed by wet-lab experiments such as immunoblotting or mass-spectrometry, proving the relationships between proteins provided by the Petri Net simulations and ultimately, confirming or contradicting the hypothesis.

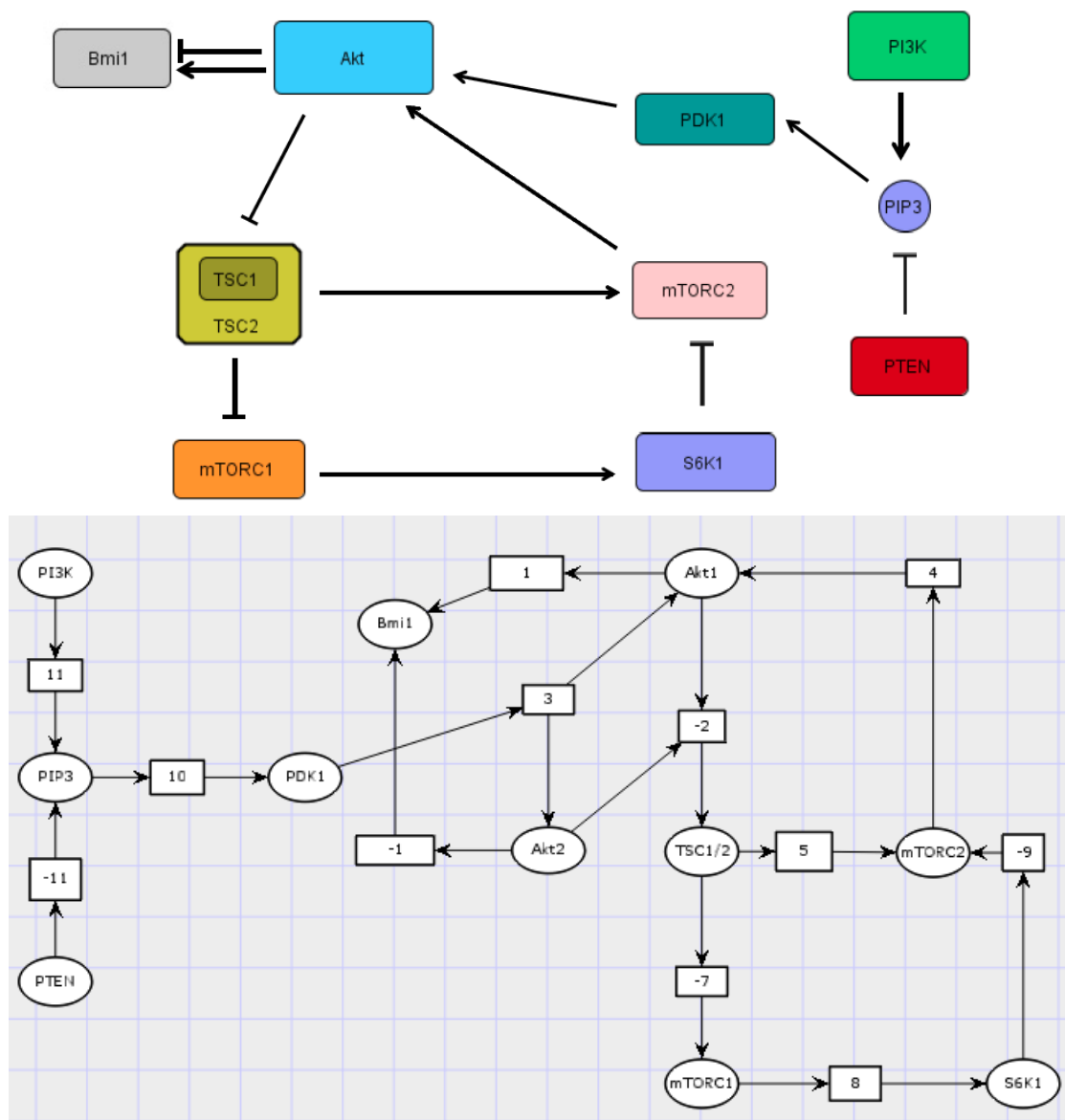


Figure 9. A network representation of Akt signaling and its corresponding Petri Net (below). Transitions marked with negative integers are used to denote inhibitory protein interactions. Bmi1 is used as a representation of Polycomb group proteins.

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6. INTERNET RESOURCES

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www.genome.ib.sci.yamaguchi-u.ac.jp

www.scienceblogs.com

www.theriac.org

7. SAŽETAK

Danas se u molekularnoj biologiji sve više pozornosti stavlja na istraživanje staničnih puteva, jer promjena u njihovoj signalizaciji može dovesti do pojave raznih i teških bolesti kao što je rak. Kombinacijom računalne snage i molekularne biologije možemo razviti dinamične mreže proteina, kojima zatim istražujemo signalizaciju staničnih puteva. Petrijeve mreže su nova metoda u računalnoj biologiji koju se može koristiti za dinamičko modeliranje staničnih regulatornih mreža. U ovom radu opisan je princip rada Petrijevih mreža i osnovne vrste Petrijevih mreža, te je dan primjer kako se Petrijeve mreže mogu koristiti za istraživanje bioloških problema.

8. SUMMARY

In comparison to single molecular interactions, more and more emphasis is being given to the signaling of cellular pathways, as their alteration can lead to outcomes such as cancer. Combining computational power with molecular biology is used to develop dynamic networks of proteins, which are then used to explore the network signaling. Petri Nets are a novel tool in bioinformatics that can be used for the dynamic modeling of cellular regulatory networks. This work describes the methodology of Petri Nets and the types of Petri Nets, and additionally, gives an example on how they can be applied in biological research.